Synthesis and stability of boron cage derivatives for use as cancer targeting agents

Oral Presentation Outline

A Senior Honors Thesis

Presented in Partial Fulfillment of the Requirements for graduation with distinction in Chemistry in the undergraduate college of The Ohio State University

By

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Abstract:

The focus of this research has been to investigate whether a boron cage derivative composed of iodine would be a thermodynamically stable compound that could be incorporated into monoclonal antibodies. These monoclonal antibodies would be used to diagnose and treat breast cancer. First, $Cs_2[B_{10}I_{10}]$ was successfully synthesized. In future studies, this compound will be mixed with rate plasma and peroxidase as a way to test its stability in physiologic conditions. Second, multiple attempts were made to synthesize $[NH(Et)_3][NMe_4][B_{10}H_9NCS]$. This is a boron compound with a linking component, isothiocyanate. Having a linking component would allow the boron compound to attach to the antibody. Unfortunately, it is not certain that $[NH(Et)_3][NMe_4][B_{10}H_9NCS]$ was synthesized. Rather, it is believed a mixture of products with the key components: isothiocyanate and a boron cage were created. Future research will continue to synthesize this product and eventually attach iodine to the boron cage. All products synthesized were verified with ^{1}H NMR, ^{13}C NMR, ^{11}B NMR, and Infrared Spectroscopy.

Introduction:

Breast cancer is a life-altering disease that can affect both men and women. Looking at statistics from the American Cancer Society, the prevalence of breast cancer has risen and fallen since the late nineteen-seventies. However, breast cancer has become the second most deadly cancer for American women. The American Cancer Society estimated that in the year 2009, 40,170 women and 1,910 men would die from this form of cancer. For several decades, chemotherapy has been the most recognized form of treatment. While this form of therapy is beneficial to some patients, cancer cells can build tolerance to a particular treatment with time which causes the body to become immune to the treatment[1]. Scientists and physicians have

researched other forms of therapy for breast cancer patients and have found that some of the most effective treatments result from earlier diagnosis, surgical ablation, and a combination of chemotherapeutic agents and radiation[2]. By mixing a multitude of treatment options the body may not become resistant to a particular therapy.

Recently, attention has been directed toward targeted therapies, in particular radioactive monoclonal antibodies (mAbs). Antibodies have been considered to have great potential as anticancer agents because they can seek specific microbes and cancer cells. In radioactive monoclonal antibodies, a radioactive compound is bonded to an antibody. This combination allows the antibody to target and destroy the tumor. The radioactive ligand also helps to destroy the tumor[3]. The initial benefits of radioactive monoclonal antibodies were seen in patients with B-cell lymphoma. In 1997, the anti-CD20 monoclonal antibody, rituximab, was approved by the Food and Drug Administration for the treatment of β-cell lymphoma. Within a little over a year, 40% of patients treated with anti-CD20 monoclonal antibody responded positively to the therapy[4, 5]. The same benefits are also idealized for patients with breast cancer.

Studies have already begun to focus on using monoclonal antibodies as a form of therapy for breast cancer patients. In patients who have tumors that overexpress the transmembrane protein, HER-2, Herceptin has been chosen for the monoclonal antibody. Additionally, the anti-body, Avastin, has also demonstrated potential in breast cancer therapy[3, 6]. However, there have been difficulties with the radioactive ligand. The "Gold Standard" for targeted radiotherapy is Iodine because it has properties widely useful for diagnosis and therapy. I^{131} has been used for *in vivo* therapeutics, and I^{125} has been used for *in vivo* diagnostics. The success of radioiodine has been seen in long-term patient survival. Currently, all radioiodinated mAbs have attached the iodine atom to carbon, in particular the ortho position of a phenol group

in the case of the Bolton-Hunter Reagent[7]. Yet, the carbon-iodine bond is not highly stable, neither thermodynamically nor biochemically[8]. This raises a safety concern for patients being treated with radioiodinated mAbs. Free iodine has the potential to cause hyperthyroidism[7].

The desired radioactive compound should consist of three important characteristics for successful incorporation with the monoclonal antibody: 1. There should be a protein-binding functionality allowing for facile, covalent incorporation into mAbs without adversely affecting the latter's conformation or *in vivo* receptor targeting; 2. It should be stable under physiological conditions; 3. Ideally, it should have the capacity for incorporating multiple radiohalogens into a single ligand, prior to or subsequent to protein binding, which would permit its use for diagnosis and therapy[7].

With knowledge of these characteristics, Dr. Sheldon Shore and Dr. Albert Soloway have proposed that the radioactive ligand be constructed with a boron-iodine bond. Their proposal is based on the knowledge that the boron-iodine bond has twice the thermodynamic stability of the carbon counterpart[7]. Therefore, the boron-iodine bond would not be expected to readily cleave under physiologic conditions and would hopefully avoid exposing patients to the possibility of developing hyperthyroidism. In particular, Dr. Shore and Dr. Soloway have selected the radioactive ligands be constructed with a cage composed of ten boron atoms and iodine molecules attached to the boron atoms. The radiolabel portion will be a decahydrodecaborate cage ($B_{10}H_{10}^{2-}$) because previous studies have shown that the anion was readily iodinated under low temperatures. The link between the radioactive atom and the antibody will be Isothiocyanate (NCS). It was chosen for two main reasons: 1. it has widespread use in fluorescein isothiocyanate for the fluorescence labeling of cells; and 2. iodination can occur in its

presence without any chemical transformation of the isothiocyanate group or alteration in its binding characteristics[7, 8].

My research was focused on synthesizing $B_{10}I_{10}^{2-}$ and $B_{10}I_{10}^{2-}$ NCS. ^{1}H NMR, ^{13}C NMR, and ^{11}B NMR were examined to ensure that the appropriate products were attained.

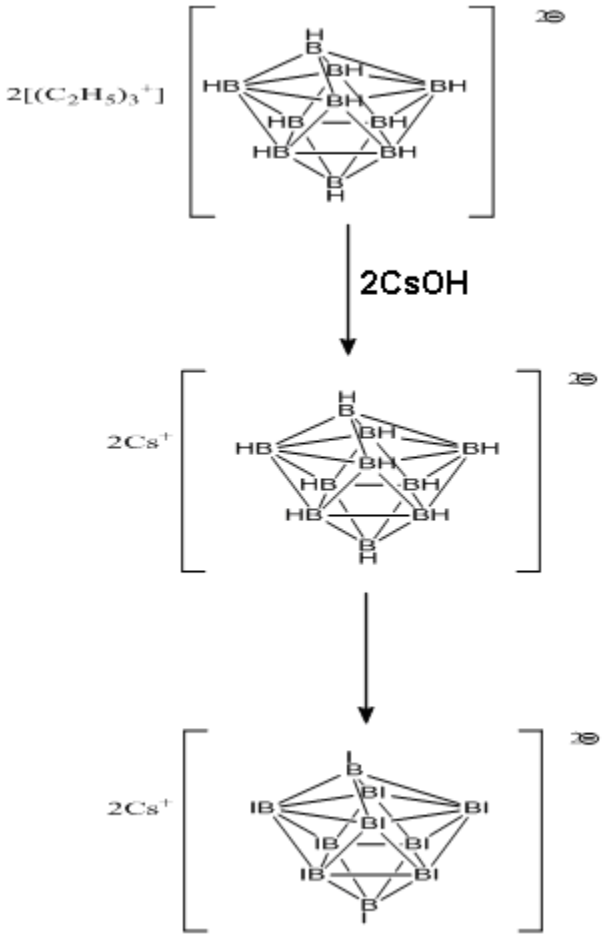
Experimental:

Equipment and materials

Standard vacuum line techniques were used for drying solid products and transferring solvents. The NMR spectra were obtained from ^{1}H NMR (250.1 MHz), ^{13}C NMR (62.9 MHz), and ^{11}B NMR on a Bruker DPX-250 spectrometer at 80.25 MHz. Boron spectra were externally referenced to $BF_3 \cdot OEt_2$ in C_6D_6 (δ=0 ppm).

Reagents used for this research project were as follows: xylene (Sigma Aldrich), triethylamine (Sigma Aldrich), isopropyl alcohol (Fisher Scientific), diethyl ether (Fisher Scientific), ethyl alcohol (Decon Laboratories Inc.), cesium hydroxide (Alfa Aesar), aniline (Sigma Aldrich), sodium nitrite (Sigma Aldrich), tetrafluoroboric (Alfa Products), sodium hydrosulfite (J. T. Baker Chemical Company), tetramethylammonium chloride (Aldrich), hydrochloric acid (12.1 M, Fisher Scientific), potassium hydroxide (Mallinckrodt Chemicals), acetonitrile (Mallinckrodt Chemicals), 1, 1' Thiocarbonyldiimidazole (Aldrich), and Iodinemonochloride (Aldrich).

Table 1: Synthesis outline for $B_{10}I_{10}^{2-}$:



Synthesis of Bis(Triethylammonium) Decahydrodecaborate

$$B_{10}H_{14} \xrightarrow[\text{Xylene}]{100 \pm 5°C,\ 3\ hrs} [(C_2H_5)_3N]_2B_{10}H_{12} \xrightarrow{130°C,\ 5\ hrs} [HN(C_2H_5)_3]_2B_{10}H_{10} \quad \textbf{(1)}$$

The synthesis of bis(triethylammonium) decahydrodecaborate for this project essentially followed the methods of Hawthorne and Olsen[9]. $B_{10}H_{14}$ (5.5 g, 45.0 mmoles, sublimed at 70-75°C under high vacuum) was dissolved in xylene (100 mL) in a 1-L three-necked flask equipped with a mechanical stirrer, a thermometer, a reflux condenser, and a heating mantle. The flask was flushed with nitrogen and 16.5 mL of triethylamine was added in

small amounts over a period of two minutes. The reaction stirred and remained heated (100 ± 5 °C) for three hours under nitrogen. During this stage, $[(C_2H_5)_3NH]_2B_{10}H_{10}$ is formed together with covalent $[(C_2H_5)_3N]_2B_{10}H_{12}$. Next, the temperature was raised to 130°C and continued to be stirred for an additional five hours under nitrogen. The solution was then cooled to room temperature and filtered. The covalent $[(C_2H_5)_3N]_2B_{10}H_{12}$ is converted to ionic $[(C_2H_5)_3NH]_2B_{10}H_{10}$ during the reflux period.

At this step, the product was a pale yellow solid. It was washed five times with 50 mL portions of isopropyl alcohol which transformed the product into a cream color. The compound was dried on the filter stand for 12 hours. Next, the product was recrystallized in water at 100 ± 5°C. Ethanol was added to the solution until a solid precipitated. The solution was cooled for several minutes in an ice-bath.

Finally, the compound was filtered under vacuum filtration. The filtrate was mixed with diethyl ether until a white solid precipitated. An ^{11}B NMR was taken of the product rinsed with ethanol and the solid that precipitated from diethyl ether. The compound, $[(C_2H_5)_3NH]_2B_{10}H_{10}$, rinsed with diethyl ether was found to be more pure and a higher yield (98%) compared to the product washed with only ethanol (95% yield).

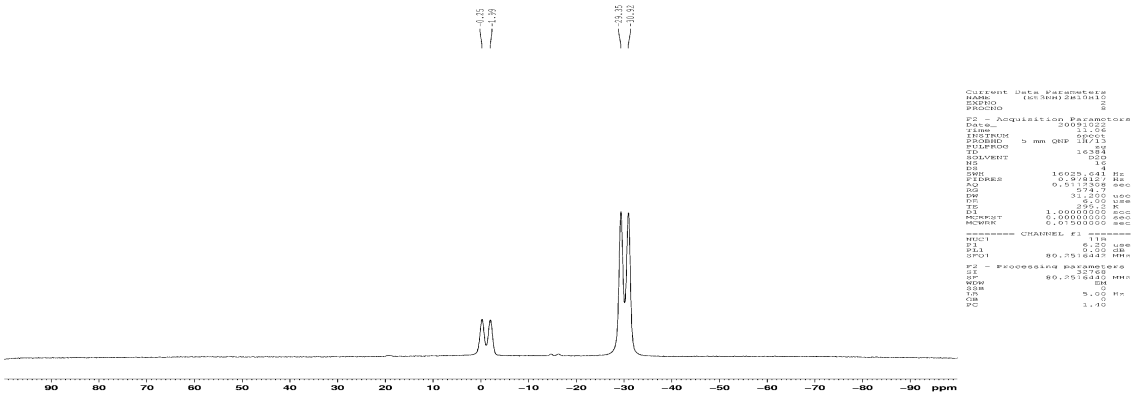


Figure 1. NMR Results for $[(C_2H_5)_3NH]_2B_{10}H_{10}$ (in D_2O)

Synthesis of $Cs_2B_{10}H_{10}$

$$[(C_2H_5)_3NH]_2 + 2CsOH \rightarrow Cs_2B_{10}H_{10} + 2[N(Et)_3] + 2H_2O \quad \textbf{(2)}$$

$K_2B_{10}H_{10}$ (2.3 g) was dissolved in D_2O (~50 mL). CsOH was added to the solution until no solid precipitated. The compound was filtered and washed with cold water. Finally the compound was dried in the vacuum system for three days.

Synthesis of $Cs_2B_{10}I_{10}$

$$Cs_2B_{10}H_{10} + 20ICl \longrightarrow Cs_2B_{10}I_{10} + 10HCl \quad \textbf{(3)}$$

Iodinemonochloride (3g) was mixed with $Cs_2B_{10}H_{10}$ (1g) in acetonitrile (20 mL) until a color change was noticed. The NMR spectrum indicated formation of $Cs_2B_{10}I_{10}$.

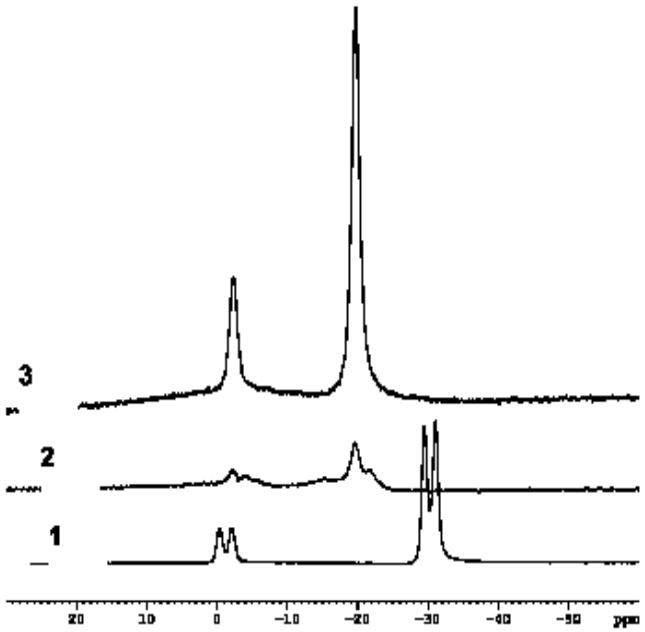
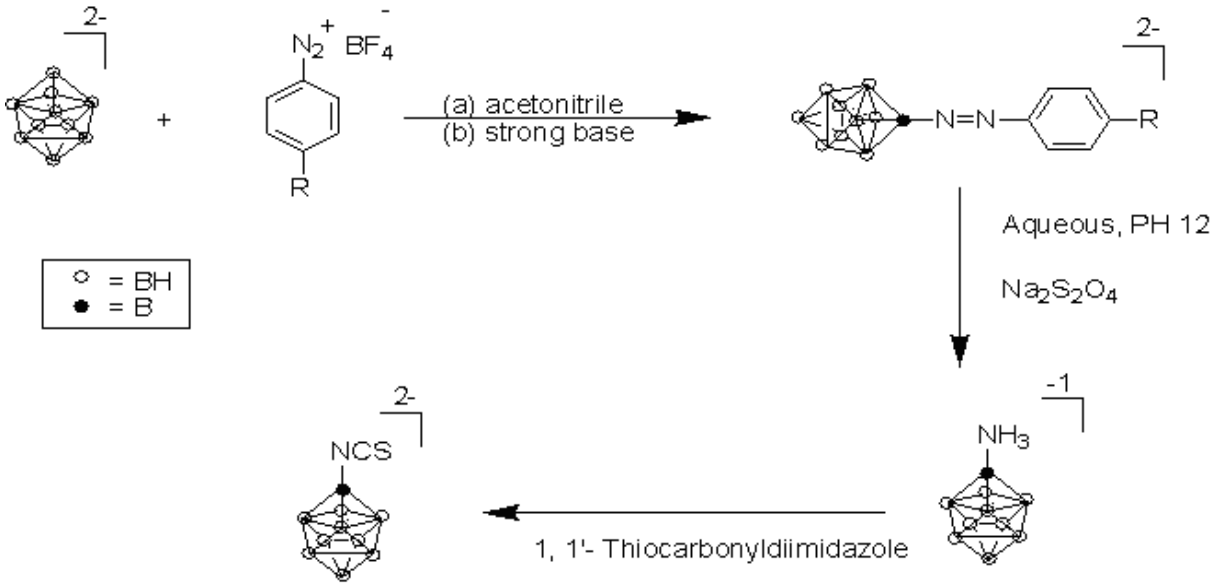


Figure 2: ^{11}B NMR Results for (1) $Cs_2B_{10}H_{10}$, (2) Intermediate for Synthesis of $Cs_2B_{10}I_{10}$, and (3) $Cs_2B_{10}I_{10}$ (in D_2O)

Table 2: Synthesis outline for Isothiocyanato-closo-Nonahydrodecaborate[7]:



Target compound 1

Synthesis of Bis(Triethylammonium) Decahydrodecaborate

$$B_{10}H_{14} \xrightarrow[\text{Xylene}]{100 \pm 5°C,\ 3\ hrs} [(C_2H_5)_3N]_2B_{10}H_{12} \xrightarrow{130°C,\ 5\ hrs} [HN(C_2H_5)_3]_2B_{10}H_{10} \quad \textbf{(4)}$$

Reaction five followed the procedure outlined in reaction 1. However, there were different amounts of starting materials: $B_{10}H_{14}$ (4.6 g, 37.7 mmoles), xylene (100 mL), and triethylamine (13.8 mL). Ultimately, Bis(triethylammonium)decahydrodecaborate (4.17 g) was formed. See Figure 1 for ^{11}B NMR results for $[(C_2H_5NH)_3]_2B_{10}H_{10}$

Synthesis of $K_2B_{10}H_{10}$

$$[HN(C_2H_5)_3]_2B_{10}H_{10} \xrightarrow[\text{Ion Exchange Column}]{H_3O^+} [H_3O]_2B_{10}H_{10} \xrightarrow{2KOH} K_2B_{10}H_{10} \quad \textbf{(5)}$$

Bis(triethylammonium)decahydrodecaborate (4.17 g, 12.9 mmoles) was dissolved in distilled water (50 mL) and passed through an acid exchange column. Distilled water (100 mL) was added to the column until the pH was neutral. This indicated that the desired product ($[H_3O^+]_2B_{10}H_{10}$) had passed through the column. Potassium hydroxide was added to the solution

until there was a neutral pH as indicated by Hawthorne and Olsen[9]. The solvent was evaporated and the product, $K_2B_{10}H_{10}$ (2.08 g), remained.

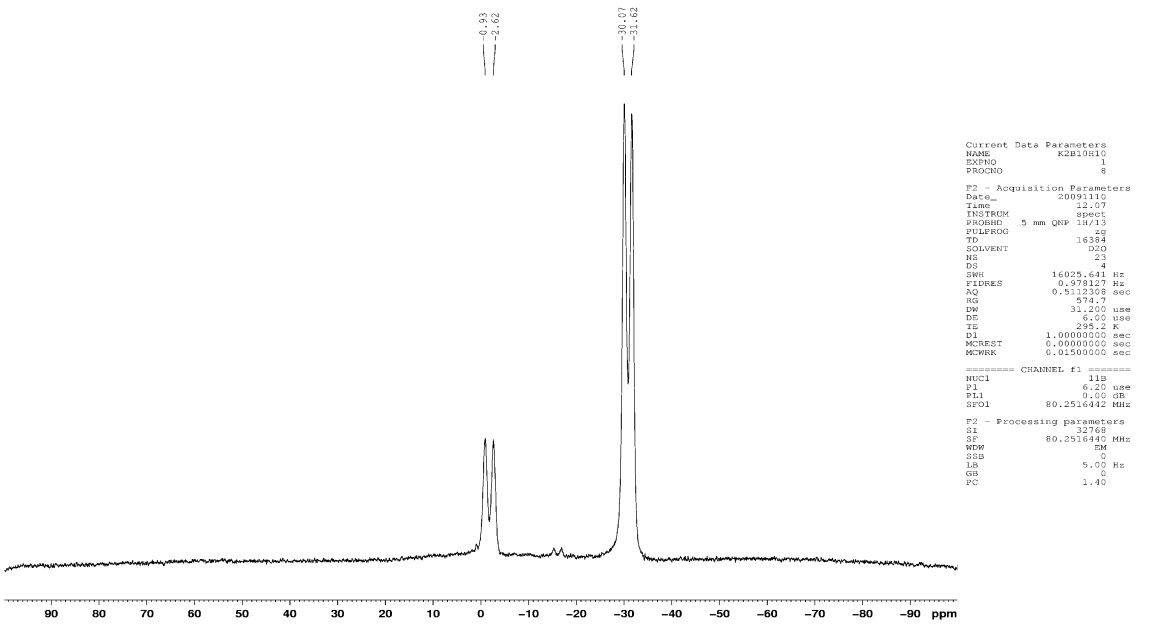


Figure 3: ^{11}B NMR Results for $K_2B_{10}H_{10}$ (in D_2O)

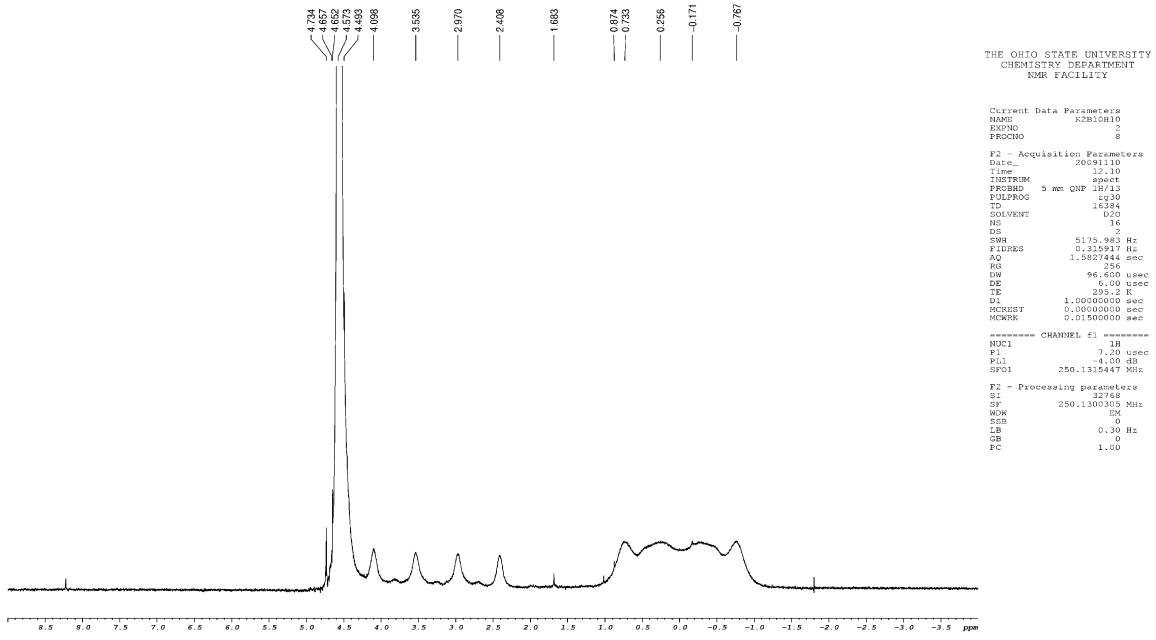


Figure 4: ^{1}H NMR Results for $K_2B_{10}H_{10}$ (in D_2O)

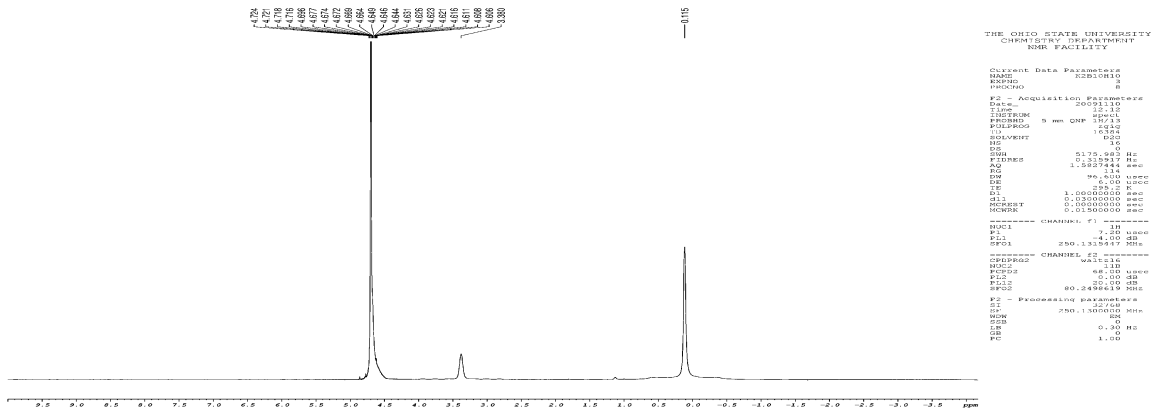


Figure 5: $^{1}H\{^{11}B\}$ NMR Results for $K_2B_{10}H_{10}$ (in D_2O)

Synthesis of $BF_4[N_2C_6H_5]$ (Benzenediazonium tetrafluoroborate)

$$1.\ NaNO_2 + HCl \xrightarrow{0°C} HNO_2$$

$$2.\ HNO_2 + C_6H_5NH_2 \xrightarrow{HBF_4,\ 0°C} [C_6H_5N_2]^+BF_4^- \qquad \textbf{(6)}$$

Aniline (3g, mmoles) was dissolved in HCl (10 mL), and the mixture was cooled to 0ºC as described by Cox and Kumamoto[10]. $NaNO_2$ (0.25 g, mmoles) was dissolved in H_2O (10 mL) and added to the cooled mixture and stirred for 20 min. After stirring, HBF_4 (tetrafluoroboric acid) was added to the mixture to form a precipitate (2.04 g, 11 mmoles).

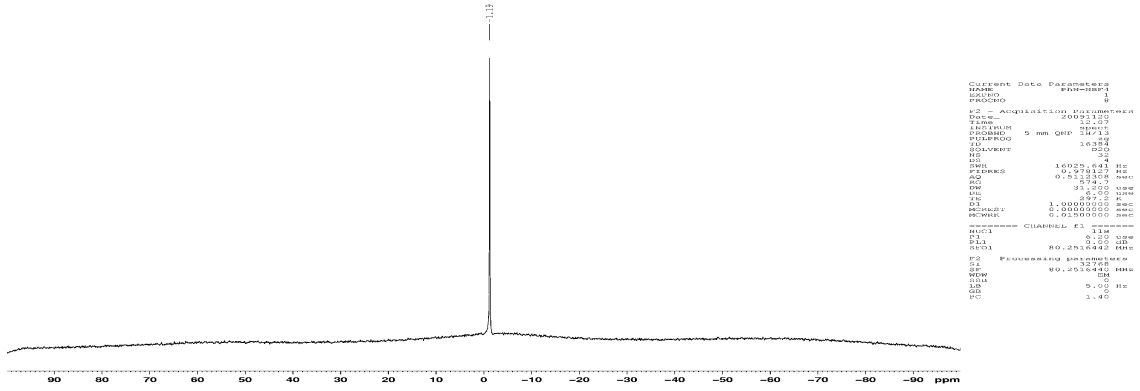


Figure 6: ^{11}B NMR Results for $C_6H_5N_2BF_4$ (in CD_3CN)

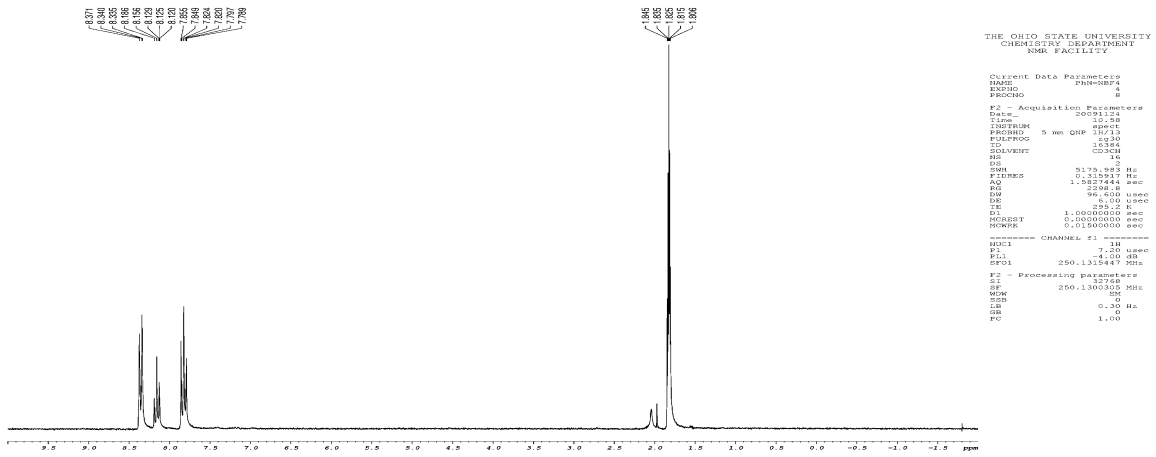


Figure 7: ^{1}H NMR Results for $C_6H_5N_2BF_4$ (in CD_3CN)

Synthesis of *$K[B_{10}H_9N_2C_6H_5]$*

$$K_2B_{10}H_{10} + C_6H_5N_2BF4 \rightarrow K[B_{10}H_9N_2C_6H_5] \qquad (7)$$

Following the procedure outlined by Hawthorne and Olsen[9], $K_2B_{10}H_{10}$ (2.08 g, 11 mmoles) was dissolved in acetonitrile (200 mL). Benzenediazonium tetrafluoroborate (2.04 g, 11 mmoles) was combined with acetonitrile (35 mL), and added dropwise to the $K_2B_{10}H_{10}$ solution at -35ºC with stirring. The solution remained stirring for 30 min and was brought to room temperature. While stirring, the solution turned from a yellow color to a deep purple. Next, the solution was concentrated to about 30 mL and filtered. The filtrate was evaporated and dried under the vacuum system, whereupon the yield was 2.09 g (7.97 mmoles) and the NMR indicated presence of the desired product, $K[B_{10}H_9N_2C_6H_5]$.

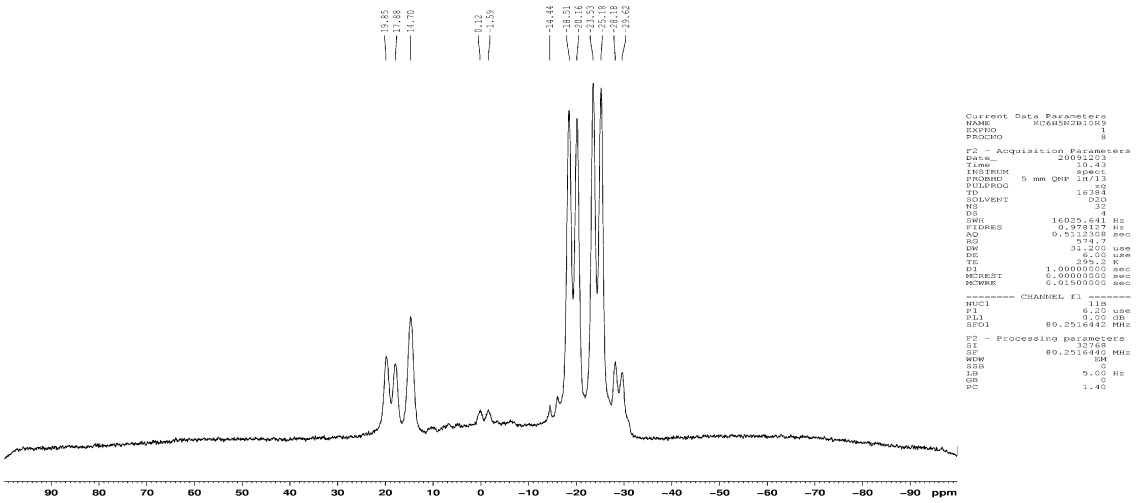


Figure 8: ^{11}B NMR Results for K[$B_{10}H_9N_2C_6H_5$] (in CD_3CN)

Synthesis of [NMe_4][$B_{10}H_9NH_3$]

$$K[B_{10}H_9N_2C_6H_5] + Na_2S_2O_4 \xrightarrow{NMe_4Cl} [NMe_4][B_{10}H_9NH_3] \quad \textbf{(8)}$$

$K[B_{10}H_9N_2C_6H_5]$ (2.09 g, 7.97 mmoles) and 2.04 g of tetramethylammonium chloride were dissolved in 52.36 mL of distilled water. The pH of the solution was adjusted to 12 by the addition of KOH pellets. After stirring the solution for 30 min, HCl was added until the pH was about 1. The solution stirred for an additional 15 min and then was filtered. (*However, the addition of tetramethylammonium chloride in the initial step of this synthesis was incorrect according to the procedure outlined by Hawthorne and Olsen[9]. Instead, solid $Na_2S_2O_4$ should have been added in the initial step and tetramethylammonium chloride should have been added to the filtrate).

In an attempt to recover $K[B_{10}H_9N_2C_6H_5]$, the solid that had been filtered was recombined with the filtrate. While stirring this solution, the pH was adjusted to about 12 with the addition of KOH pellets and $Na_2S_2O_4$ (2.04 g). The basic solution was stirred for 30 minutes. Following the correct procedure outline by Hawthorne and Olsen[9], HCl was added to the basic

solution until the pH was about 1. The solution was stirred for 15 min and filtered. An excess amount of tetramethylammonium chloride was added to the filtrate, and this solution "B" was then allowed to stand for several days to allow solid to precipitate from solution. Solution "B" was filtered, and the yellow solid was dried under vacuum for a day. The yellow solid (0.32 g) was dissolved in 50 mL of acetonitrile, stirred for 30 min, and then was filtered. The filtrate was evaporated, whereupon NMR results indicated the presence of the desired product, $[NMe_4][B_{10}H_9NH_3]$. The product was dried under the vacuum system to ensure that water and acetonitrile were completely removed before proceeding to the next step in the outlined synthesis (Figure 2). Ultimately, 0.029 g of the solid, $[NMe_4][B_{10}H_9NH_3]$ was collected.

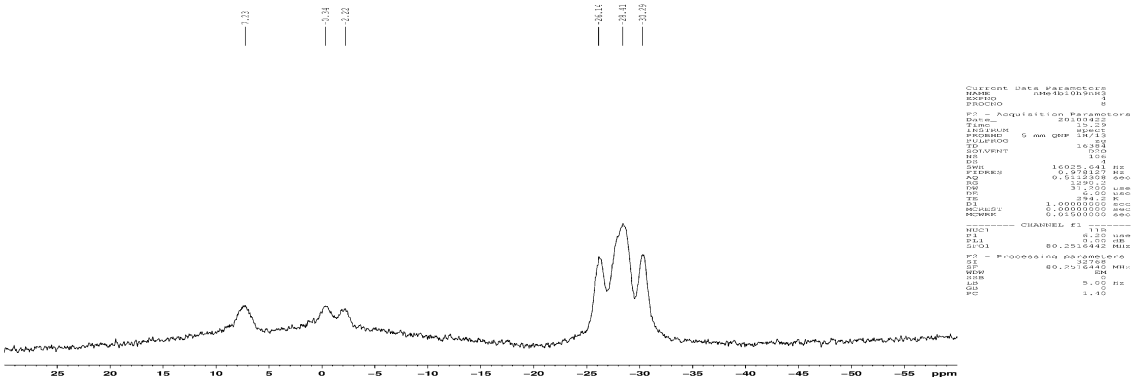


Figure 9: ^{11}B NMR Results for $[NMe_4][B_{10}H_9NH_3]$ (in CD_3CN)

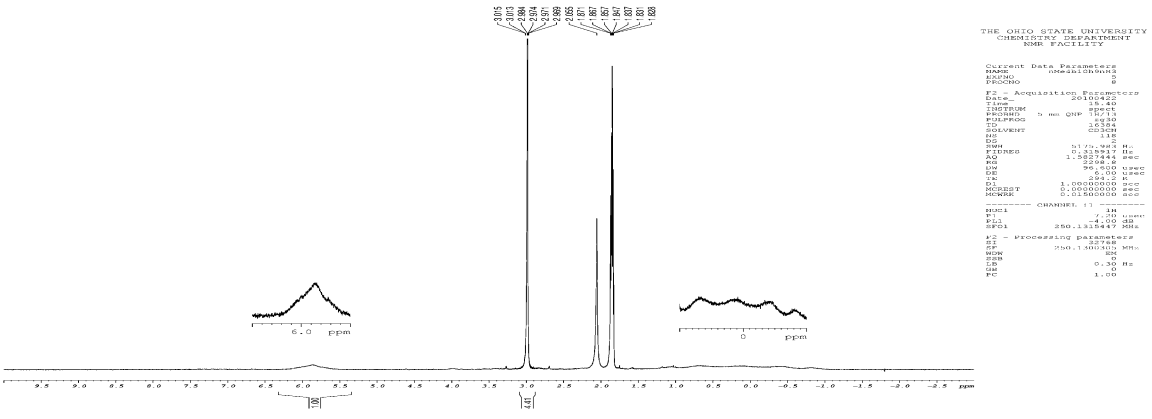


Figure 10: ^{1}H NMR Results for $[NMe_4][B_{10}H_9NH_3]$ (in CD_3CN)

Synthesis of [NMe$_4$][HN(Et)$_3$][B$_{10}$H$_9$NCS]

$$[NMe_4][B_{10}H_9NH_3] + N(CH_2CH_3)_3 + \text{1,1 Thiocarbonyldiimidazole} \longrightarrow [HN(Et)_3][NMe_4][B_{10}H_9NCS] + \text{Unknown Compound} \quad \textbf{(9)}$$

The solid, [NMe$_4$][B$_{10}$H$_9$NH$_3$] (0.029 g, 0.14 mmoles), was mixed with triethylamine (0.014 g), 1,1' Thiocarbonyldiimidazole (0.025 g) in acetonitrile (20 mL). The solution was stirred for twenty-four hours. A ^{11}B NMR, ^{1}H NMR, ^{13}C NMR, and IR helped to conclude that a mixture of products had been formed. These results are described in the following Results and Discussion section.

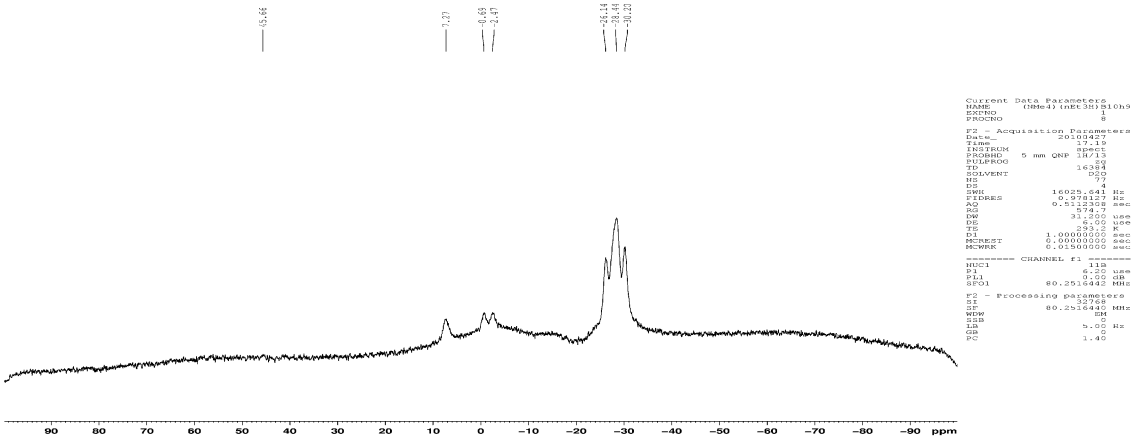


Figure 11: ^{11}B NMR Results for [NMe$_4$][N(Et)$_3$H][B$_{10}$H$_9$NCS] ((in CD$_3$CN, after 1 hr. of reaction)

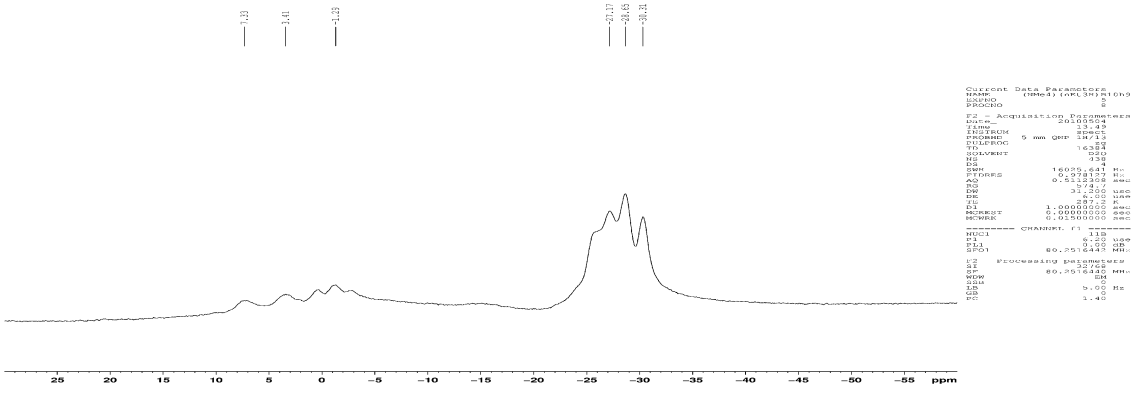


Figure 12: ^{11}B NMR Results for EXPERIMENTALLY Determined B$_{10}$H$_9$NCS (in CD$_3$CN)

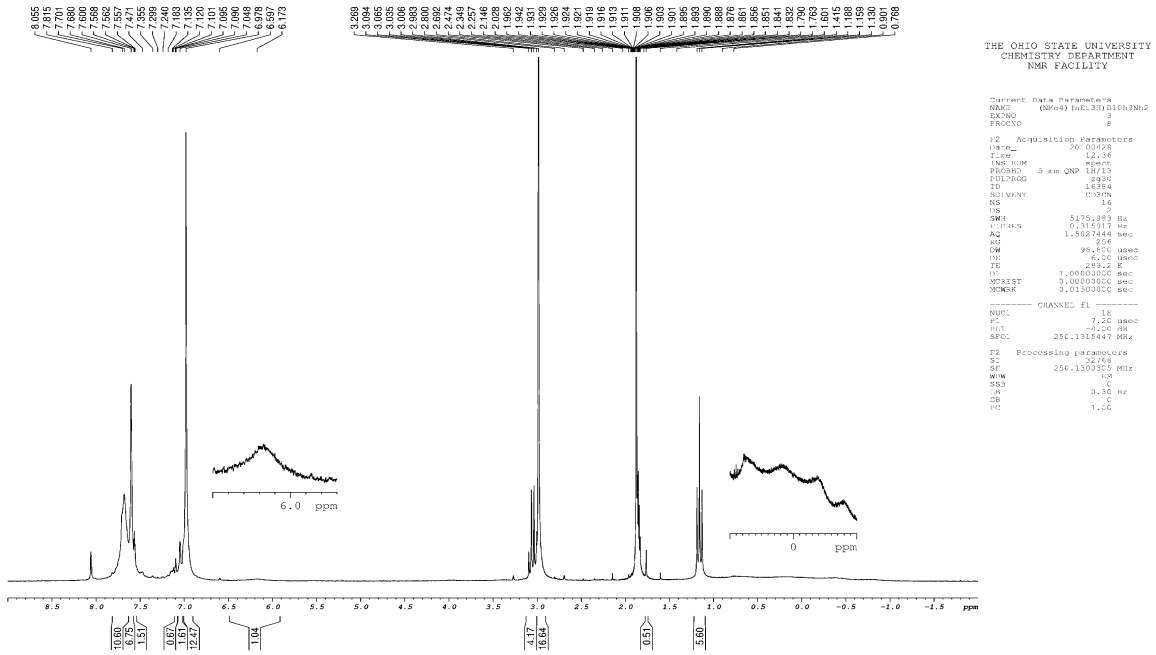


Figure 13: ^{1}H NMR Results for $B_{10}H_9NCS$ (in CD_3CN)

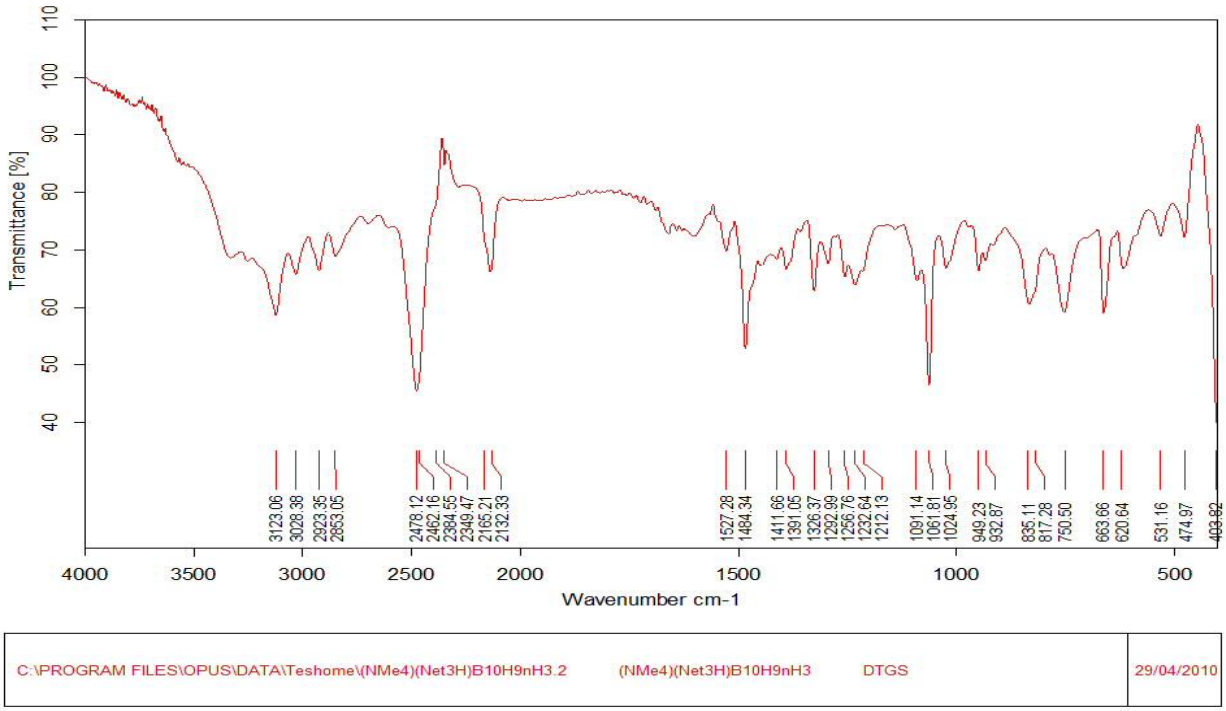


Figure 14: IR Results for $B_{10}H_9NCS$ (NaCl, neat)

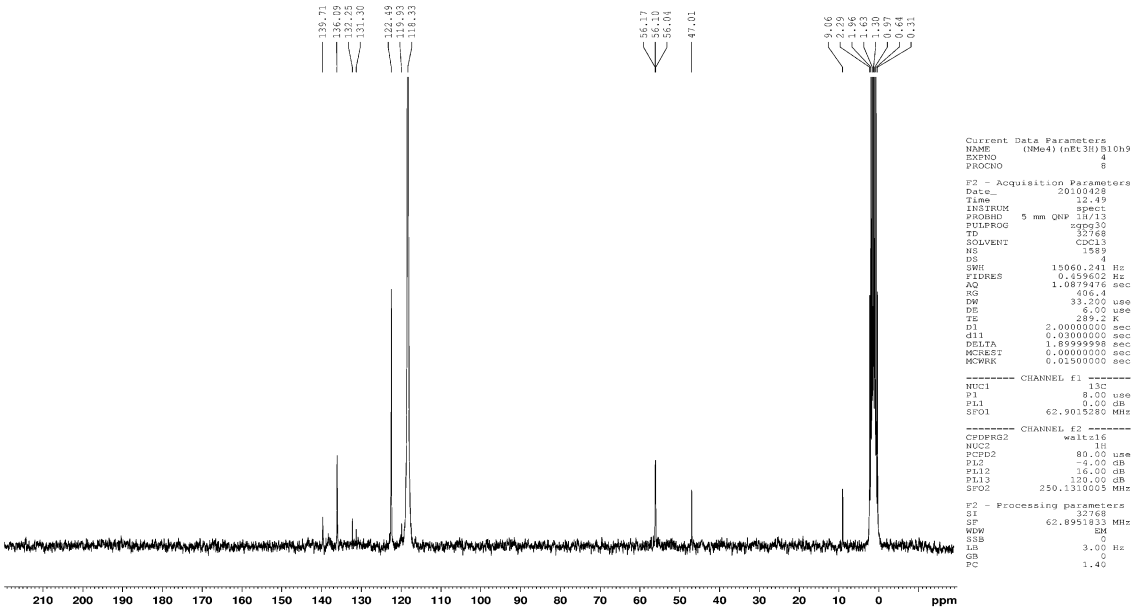


Figure 15: ^{13}NMR Results for $[NH(Et)_3][NMe_4]B_{10}H_9NCS$ (in CD_3CN)

Results and Discussion:

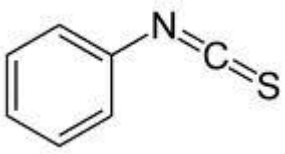
Synthesis of Isothiocyanato-closo-nonahydrodecaborate

There had been no literature to describe the synthesis of Isothiocyanato-closo-nonahydrodecaborate. Therefore, two attempts were performed to make the desired boron based compound (Illustrated as target compound 1 in Table 2).

In the first attempt, $[NMe_4][B_{10}H_9NH_3]$ was successfully prepared using the outlined procedure in Table 2. In an attempt to convert the compound to the desired product, the reagent 1, 1'- Thiocarbonyldiimidazole and the solvent, acetonitrile, were mixed with $[NMe_4][B_{10}H_9NH_3]$. The mixture was stirred for several hours and the acetonitrile was evaporated and dried under the vacuum system for a day. However, the NMR showed no formation of the Isothiocyanato-*closo*-nonahydrodecaborate. Rather, the NMR indicated that none of the initial product had reacted.

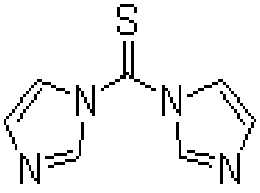
However, the second attempt to create Isothiocyanato-*closo*-nonahydrodecaborate was more successful. Again, $[NMe_4][B_{10}H_9NH_3]$ was prepared. Different from the first attempt, triethylamine was added along with the 1, 1'- Thiocarbonyldiimidazole and acetonitrile to the $[NMe_4][B_{10}H_9NH_3]$ solid. The mixture (Noted as Reaction 8 in the experimental section) was stirred, and a ^{1}H NMR, ^{13}C NMR, and ^{11}B NMR were taken after twenty-four hours of mixing. Based on the shift in peaks after twenty-four hours of mixing compared to the NMR data for $[NMe_4][B_{10}H_9NH_3]$, there appears to be a mixture of products that are believed to be the beginning stages of the formation of Isothiocyanato-*closo*-nonahydrodecaborate. Additionally, an IR spectroscopy showed evidence of the desired product being produced.

In the ideal situation, the desired product will be formed from the nucleophilic NH_2 group on the $[HN(C_2H_5)3][NMe_4][B_{10}H_9NH_2]$ reacting with the carbon on the 1, 1' – thiocarbonyldiimidazole. From this reaction, two imidazole groups will detach from 1, 1' – thiocarbonlydiimidazole and what will remain is the desired product. It is not known where the peaks for the product will be located on both the proton and carbon-13 NMR's since this synthesis has not been attempted before. Literature values for phenyl isothiocyanate, imidazole, and 1, 1' - Thiocarbonyldiimidazole provided an idea to where the peaks for the products would be expected[11]. The figures below are these three compounds.



Phenyl Isothiocyanate

Imidazole



1,1' - Thiocarbonyldiimidazole

If the desired product had formed, three peaks on ^{13}C-NMR would be seen. There would be two peaks from the imidazole groups and one peak from the isothiocyanate group. However, examining the ^{13}C-NMR for the reaction, there were six peaks seen from 122.04 ppm – 139.70 ppm. Two peaks were seen at 136.09 ppm and 122.04 ppm. The ratio of the peaks were 1:2. These peaks are believed to show the formation of an imidazole. This conclusion was based on the literature data that shows an imidazole group will be seen at 135.35 ppm and 121.88 ppm. Also, the peaks will be in a 1:2 ratio. Additionally, there is a small peak seen at 138 ppm which is believed to be the isothiocyanate group. This conclusion was based on literature values for phenyl isothiocyanate showing that a less intense peak will be seen at 135.44 ppm. However, it is unclear from what compounds the peaks at 139.70, 132.26, and 131.30 ppm stem. Yet, examining the ^{11}B-NMR, there were a mixture of products that formed. Thus, the three mystery peaks are likely to be stemming from the mixture of products.

The ^{11}B NMR is an important component in determining whether or not the desired product formed, because the NMR should not be different for the conversion of $[NMe_4][B_{10}H_9NH_3]$ to $[HN(Et)_3][NMe_4][B_{10}H_9NCS]$. This is due to no reaction expected to occur on the boron cage. However, the results from ^{11}B NMR illustrate that a chemical reaction occurred that affected the structure of the boron cage. From the ^{11}B NMR (Figure 11) for $[NMe_4][B_{10}H_9NH_3]$, it is known that there should be a triplet from -26.14 ppm to -30.20 ppm, a doublet from -0.69 ppm to -2.47 ppm, and a singlet at 7.27 ppm. However, for the NMR taken on reaction 8 (Figure 12), additional peaks are seen on the triplet and throughout -0.89 ppm to 10 ppm (Figure 12). Thus, the extra peaks indicate that a mixture of products has formed. Yet, it is not clear as to what products were created.

From the proton NMR (Figure 13), there were two peaks at 7.7 ppm and 6.97 ppm, which is the same as the literature values for an imidazole group. Additionally, there were two peaks at 8 and 7.5 ppm which are similar to the literature values for 1, 1' – thiocarbonlydiimidazole.

An IR (Figure 14), showed a peak at 2478.12 cm^{-1} due to the boron-hydrogen stretch. Similarly, a peak was seen at 2165.21 cm^{-1}, which indicated the stretch for the isothiocyanate group. The literature value for the isothiocyanate group on phenyl isothiocyanate is seen at 2087 cm^{-1}.

Interestingly, when comparing the ^{1}H NMR for $[NMe_4][B_{10}H_9NH_3]$ (Figure 10) to $[HN(Et)_3][NMe_4][B_{10}H_9NCS]$ (Figure 13), the ratio of the peaks for the amine to NMe_4 has changed from 1:4 to 1:16. The change in the ratio between these compounds shows that an estimated 75% of the amine has reacted. This is an exciting discovery since it is desired for the amine to attack the carbon on 1, 1' thiocarbonyldiimidazole after losing a proton from triethylamine. Additionally, the ^{1}H NMRs show that one of triethylamine's peaks has shifted from 2.425 to 3 ppm, which provides evidence that it has extracted a proton from the amine.

Lastly, a peak at 172.88 ppm which is the carbon-sulfide bond on 1,1' thiocarbonyldiimidazole disappeared after twenty-four hours of mixing (Figure 15).

Conclusions:

After examining the NMR's and IR's taken on reaction 8 compared to the NMR data for $[NMe_4][B_{10}H_9NH_3]$, it has been concluded that a compound with the isothiocyanate group and boron cage has formed. It is believed that the NH_2 attached to $B_{10}H_9$ has attacked 1, 1' – Thicarbonlydiimidazole and consequently caused some of the imidazole groups to detach. This conclusion is supported by the peaks for imidazole and a shift in the peak values for 1, 1' – Thicarbonlydiimidazole evident on both the proton and carbon-13 NMR's. Additionally, the IR

showing the isothiocyanate peak supports that a compound with the isothiocyanate group has formed. Additionally, since the peaks shifted for the triethylamine, it is believed that this reagent removed a proton from the amine group on the boron cage that allowed NH_2 to attack 1, 1' – Thicarbonlydiimidazole. Finally, it is not known what chemistry caused the carbon-sulfide peak to disappear at 172.88 ppm (Figure 15). Yet, having the peak disappear provides support that NH_2 attacked the starting reagent and consequently caused the isothiocyanate group to form.

It is unclear as to what compounds are causing the unknown peaks in the ^{13}C NMR and ^{11}B NMR. Since the estimations for the peaks seen in the NMRs and IRs are stemming from phenyl isothiocyanate, which has the isothiocyanate group attached to a benzene ring and not a boron cage, the exact values for the targeted compound will be different. Compared to the benzene ring, the boron cage has a larger electron environment that can react with the isothiocyanate group to cause the peaks to be different on the NMRs and IRs. Additionally, the reactions with phenyl isothiocyanate are performed using the solvent dichloromethane. However, the boron cage product is not soluble in dichloromethane, yet is soluble in acetonitrile. There may be side reactions using acetonitrile that may explain the unknown peaks in the ^{11}B NMRs and ^{13}C NMRs.

To further support the results from this experiment and explain the uncertainties, the procedure will be repeated. An error for this experiment was that a small sample of $[NMe_4][B_{10}H_9NH_3]$ was used to make the desired isothiocyanate product. Future syntheses will use a larger quantity of $[NMe_4][B_{10}H_9NH_3]$, such as 3 g rather than 0.029 g, in hopes that the desired product will be formed. Additionally, with a larger amount of product, a TLC is recommended to better analyze the products. Different solvents will have to be tested in order to

effectively and efficiently separate the products. Yet, this method will better indicate the exact products.

Future Work:

Synthesis of $B_{10}I_{10}$

Future work in the laboratory will be focused on testing the stability of $B_{10}I_{10}$ under physiologic conditions. Since the main goal of this project is to use the boron ligand as an effective tool with a monoclonal antibody to diagnose and treat breast cancer, future work will be centered on the safety of using this product on humans. Thus, to test the stability of this molecule under physiologic conditions, rat plasma will be chosen that contains enzymes that may destabilize the radioactive compound. Also, peroxidase will be selected to see whether it oxidizes the radioactive compound. While iodine is important for humans, too much radioactive iodine can cause greater harm to the health of humans. Thus, it will be important to find an enzyme that will not remove the iodine from the monoclonal antibody and thus allow it to effectively diagnose and treat breast cancer.

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